AG

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To:
G.E. EHRLICH (1995) LTD.
11 MENACHEM BEGIN STREET
52521 RAMAT GAN
ISRAEL

RECEIVED
07 MAY 2010
FILE No 43186
G.E. EHRLICH (1995) LTD.

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT AND THE WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference 43186	Date of mailing *(day/month/year)* 09 APR 2010 (d) **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/IL 08/00576	International filing date *(day/month/year)* 30 April 2008 (30.04.2008)
Applicant PROTALIX LTD.	

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

   Filing of amendments and statement under Article 19:
   The applicant is entitled, if he so wishes, to amend the claims of the international application (see Rule 46):

   When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

   Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes 1211 Geneva 20, Switzerland, Facsimile No.: +41 22 338 8270

   For more **detailed instructions,** see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

   ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

   ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Reminders**

   Shortly after the expiration of **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

   The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 30 months from the priority date.

   Within **19 months** from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase **until 30 months** from the priority date (in some Offices even later); otherwise, the applicant must, **within 20 months** from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

   In respect of other designated Offices, the time limit of **30 months** (or later) will apply even if no demand is filed within 19 months.

   See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the *PCT Applicant's Guide*, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/220 (January 2004) *(See notes on accompanying sheet)*

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 43186	**FOR FURTHER ACTION**	see Form PCT/ISA/220 as well as, where applicable, item 5 below.
International application No. PCT/IL 08/00576	International filing date *(day/month/year)* 30 April 2008 (30.04.2008)	(Earliest) Priority Date *(day/month/year)* 30 April 2007 (30.04.2007)
Applicant PROTALIX LTD.		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 4 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

   b. ☐ This international search report has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43.6*bis*(a)).

   c. ☒ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, see Box No. I.

2. ☐ **Certain claims were found unsearchable** (see Box No. II).

3. ☐ **Unity of invention is lacking** (see Box No. III).

4. With regard to the **title**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the **drawings**,

   a. the figure of the **drawings** to be published with the abstract is Figure No. _____

   ☐ as suggested by the applicant.

   ☐ as selected by this Authority, because the applicant failed to suggest a figure.

   ☐ as selected by this Authority, because this figure better characterizes the invention.

   b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 08/00576

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☒ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 08/00576

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07H 21/04, C07K 14/00 (2010.01)
USPC - 536/23.5, 530/350, 435/419, 435/69.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC – 536/23.5, 530/350, 435/419, 435/69.1, 435/410

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST – PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files ? 654, 652, 351, 349, 6, 35, 65, 155; USPTO Web Page; PCT Patentscope; Google Scholar; Search terms -- polynucleotide sequence, encoding lysosomal protein, ER targeting, ER retention, alpha galactoosidase, glucocerebrosidase, mannose, xylose, fusose, plant cell transfection, carrot, tobacco,

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y -- A	US 2006/0204487 A1 (SHAATIEL et al.) 14 September 2006 (14.09.2006) para [0001], [0018], [0020], [0021], [0024], [0028], [0030], [0033], [0038], [0046], [0064], [0065], [0076], [0077], [0127], [0133], [0138], [0139], [0141], [0142], [0245], [0308], [0310], Fig 7, SEQ ID NO: 8	1, 2, 8, 10-18, 20-30, 33, 37, 38, 40-47, 49, 52-55, 58-60 --- 3-5, 9, 19, 31, 32, 34, 35, 39, 48, 50, 56, 57 --- 6, 7, 36, 51
Y	US 2003/0077806 A1 (SELDEN et al.) 24 April 2003 (24.04.2003) para [0014], [0058], Fig 6, SEQ ID NO: 4	3, 9, 35, 50
Y	US 2005/0032211 A1 (SHAATIEL) 10 February 2005 (10.02.2005) para [0028], [0221], [0222], SEQ ID NOS: 1, 2, 4, 14	4, 31, 32, 34, 48, 56, 57
Y	WO 2007/005882 2 (WEISSINGER et al.) 11 January 2007 (11.01.2007) pg 1, ln 12-14; pg 4, ln 10-15; pg 5, ln 21-23, Fig 5, SEQ ID NO: 4	5, 19
Y	US2005/0281805 A1 (LEBOWITZ et al.) 22 December 2005 (22.12.2005) para [0049], [0160], [0166], [0167], [0175], [0199], Fig 30	39

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 March 2010 (16.03.2010)	09 APR 2010
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 08/00576

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,083,725 A (SELDEN et al.) 04 July 2000 (04.07.2000) col 5, ln 28-32; Fig 9, SEQ ID NO: 26	36, 51
A	US 2002/0088024 A1 (GARGER et al.) 04 July 2002 (04.07.2002) para [0051], [0062], SEQ ID NO: 10	36, 51
T	WO 2008/132743 A2 (SHAATIEL et al.) 06 November 2008 (06.11.2008) SEQ ID NOS: 17, 19	6, 7

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To: G.E. EHRLICH (1995) LTD.
11 MENACHEM BEGIN STREET
52521 RAMAT GAN
ISRAEL

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	09 APR 2010
Applicant's or agent's file reference 43186	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IL 08/00576	30 April 2008 (30.04.2008)	30 April 2007 (30.04.2007)

International Patent Classification (IPC) or both national classification and IPC
IPC(8) - C07H 21/04, C07K 14/00 (2010.01)
USPC - 536/23.5, 530/350, 435/419, 45/69.1

Applicant PROTALIX LTD.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US	Date of completion of this opinion	Authorized officer:
Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	17 March 2010 (17.03.2010)	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (July 2009)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL 08/00576

Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed.

   ☐ a translation of the international application into _____ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:

   a. (means)

      ☐ on paper

      ☒ in electronic form

   b. (time)

      ☐ in the international application as filed

      ☒ together with the international application in electronic form

      ☐ subsequently to this Authority for the purposes of search

4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL 08/00576

Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	3-7, 9, 16, 19, 21, 24, 31, 32, 34-36, 39, 41, 45-51, 56, 57	YES
	Claims	SEE CONTINUATION SHEET.	NO
Inventive step (IS)	Claims	6, 7, 36, 51	YES
	Claims	1-5, 8-35, 37-50, 52-60	NO
Industrial applicability (IA)	Claims	1-60	YES
	Claims	NONE	NO

2. Citations and explanations:

Claims 1, 2, 8, 10-15, 17, 18, 20, 22, 23, 25-30, 33, 37, 38, 40, 42-44, 52-55 and 58-60 lack novelty under PCT Article 33(2) as being anticipated by US 2006/0204487 A1 to Shaatiel et al. (hereinafter 'Shaatiel '487').

Regarding claim 1, Shaatiel '487 teaches an isolated nucleic acid sequence (para [0067], [0077], SEQ ID NO: 8) encoding a human (para [0038], [0149]) lysosomal protein (para [0067]) being contiguously linked to a C-terminal vacuolar targeting signal (para [0028], [0067]), and an N-terminal endoplasmic reticulum signal peptide (para [0023], [0028], [0128]), wherein said human (para [0038], [0149]) lysosomal protein is a human alpha-galactosidase (para [0038], [0046]).

Regarding claim 2, Shaatiel '487 teaches an isolated nucleic acid sequence (para [0067], [0077], SEQ ID NO: 8) encoding a human lysosomal protein (para [0067]) being contiguously linked to a C-terminal (para [0028]) endoplasmic reticulum retention signal (para [0127], [0141]) and an N-terminal endoplasmic reticulum signal peptide (para [0023], [0028], [0128]), wherein said human (para [0038], [0149]) lysosomal protein is a human alpha-galactosidase (para [0038], [0046]).

Regarding claim 8, Shaatiel '487 teaches the isolated nucleic acid construct of claim 1, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is human alpha-galactosidase (para [0038], [0046]).

Regarding claim 10, Shaatiel '487 teaches a nucleic acid construct (para [0133], [0141]) capable of expression in a plant cell (para [0020]) comprising the isolated nucleic acid (para [0067], [0077], SEQ ID NO: 8) of claim 1.

Regarding claim 11, Shaatiel '487 teaches a nucleic acid construct (para [0133], [0141]) capable of expression in a plant cell (para [0020]) comprising the isolated nucleic acid (para [0067], [0077], SEQ ID NO: 8) of claim 2.

Regarding claim 12, Shaatiel '487 teaches a cell (para [0033], [0139], root cells) comprising the nucleic acid construct (para [0133], [0141]) of claim 10.

Regarding claim 13, Shaatiel '487 teaches a cell (para [0033], [0139], root cells) comprising the nucleic acid construct (para [0133], [0141]) of claim 11.

Regarding claim 14, Shaatiel '487 teaches the cell of claim 13, recombinantly producing (para [0030]) said human (para [0038], [0149]) lysosomal enzyme (para [0065], [0067]).

Regarding claim 15, Shaatiel '487 teaches the cell of claim 13, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is recombinantly produced (para [0030]) so as to have at least one xylose (para [0024], [0310]) and at least one exposed mannose residue (para [0037], [0041]).

Regarding claim 17, Shaatiel '487 teaches the cell of claim 13, wherein said cell is a plant cell (para [0139], carrot cell).

Regarding claim 18, Shaatiel '487 teaches the cell of claim 17, wherein said plant cell is a plant root cell (para [0033], [0139]) consisting of a carrot cell (para [0139]).

Regarding claim 20, Shaatiel '487 teaches the cell of claim 13, wherein said cell is an Agrobacterium tumefaciens cell (para [0070]).

Regarding claim 22, Shaatiel '487 teaches a human (para [0038], [0149]) lysosomal protein (para [0067]) comprising at least one exposed mannose residue (para [0037], [0041]) and at least one fucose residue (para [0024], [0310]) having an alpha (1-3) glycosidic bond (para [0308], [0310]).

Regarding claim 23, Shaatiel '487 teaches the human lysosomal protein of claim 22, further comprising at least one xylose residue (para [0024], [0310]).

Regarding claim 25, Shaatiel '487 teaches the human lysosomal protein of claim 22, wherein said lysosomal enzyme (para [0065], [0067]) is a glucocerebrosidase (para [0021], [0076]).

SEE CONTINUATION SHEET.

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IL 08/00576

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
Claim 55 as written is dependent upon claim 54. However, the claim lacks an antecedent for the vacuolar targeting signal. Therefore, for purposes of the opinion, claim 54 is deemed to depend on laim 52.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V, No 1

NOVELTY (NO) -- 1, 2, 8, 10-15, 17, 18, 20, 22, 23, 25-30, 33, 37, 38, 40, 42-44, 52-55, 58-60

Box V, No 2

Regarding claim 26, Shaatiel '487 teaches the human lysosomal protein of claim 22, wherein said human (para [0038], [0149]) lysosomal enzyme (para [0065], [0067]) is human alpha-galactosidase (para [0038], [0046]).

Regarding claim 27, Shaatiel '487 teaches the human lysosomal protein of claim 22, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is contiguously linked to a C-terminal vacuolar targeting signal (para [0028], [0067]).

Regarding claim 28, Shaatiel '487 teaches the human lysosomal protein of claim 22, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is contiguously linked to a C-terminal vacuolar targeting signal (para [0028], [0067]) and an N-terminal endoplasmic reticulum signal peptide (para [0023], [0028], [0128]).

Regarding claim 29, Shaatiel '487 teaches the human lysosomal protein of claim 22, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is contiguously linked to a C-terminal (para [0028]) endoplasmic reticulum retention signal (para [0127], [0141]) and an N -terminal endoplasmic reticulum signal peptide (para [0023], [0028], [0128]).

Regarding claim 30, Shaatiel '487 teaches the human lysosomal protein of claim 27, wherein said vacuolar targeting signal (para [0028], [0067]) is a basic tobacco chitinase A gene vacuolar targeting signal (para [0028], [0035], [0142].

Regarding claim 33, Shaatiel '487 teaches the human lysosomal protein of claim 25, wherein said human glucocerebrosidase (para [0021], [0076]) comprises an amino acid sequence as set forth in SEQ ID NO: 8 (para [0077], claim 23, SEQ ID NO: 8).

Regarding claim 37, Shattiel '487 teaches the human lysosomal protein of claim 22, wherein said lysosomal protein (para [0067]) has a biological activity (para [0020], enzymatically active).

Regarding claim 38, Shattiel '487 teaches the human lysosomal protein of claim 37, wherein said biological activity is uptake into macrophages (para [0234], [0235]).

Regarding claim 40, Shattiel '487 teaches the human lysosomal protein of claim 37, wherein said biological activity is enzymatic activity (para [0020]).

Regarding claim 42, Shaatiel '487 teaches a pharmaceutical composition (para [0063]) comprising the human lysosomal protein of claim 22 and a pharmaceutically acceptable carrier (para [0063]).

Regarding claim 43, Shaatiel '487 teaches a plant cell preparation (para [0029], [0033], [0139]) comprising a human (para [0038], [0149]) lysosomal protein (para [0067]) comprising at least one exposed mannose residue (para [0037], [0041]) and at least one fucose residue (para [0024], [0310]) having an alpha (1-3) glycosidic bond (para [0308], [0310]).

Regarding claim 44, Shaatiel '487 teaches the plant cell preparation of claim 43, further comprising at least one xylose residue (para [0024], [0310]).

Regarding claim 52, Shaatiel '487 teaches the plant cell preparation of claim 43, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is contiguously linked to a C-terminal vacuolar targeting signal (para [0028], [0067]).

Regarding claim 53, Shaatiel '487 teaches the plant cell preparation of claim 43, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is contiguously linked to a C-terminal vacuolar targeting signal (para [0028], [0067]) and an N-terminal endoplasmic reticulum signal peptide (para [0023], [0028], [0128]).

Regarding claim 54, Shaatiel '487 teaches the plant cell preparation of claim 43, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is contiguously linked to a C-terminal (para [0028]) endoplasmic reticulum retention signal (para [0127], [0141]) and an N -terminal endoplasmic reticulum signal peptide (para [0023], [0028], [0128]).

Regarding claim 55, Shaatiel '487 teaches the plant cell preparation of claim 52, wherein said vacuolar targeting signal (para [0028], [0067]) is a basic tobacco chitinase A gene vacuolar targeting signal (para [0028], [0035], [0142].

Regarding claim 58, Shaatiel '487 teaches the plant cell preparation of claim 43, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) having at least one exposed mannose residue (para [0037], [0041]) comprises a dominant fraction (para [0248], predominantly mannose glycans) of said lysosomal protein (para [0001], [0018], protein with high mannose levels), as measured by linkage analysis (para [0245], [0306]).

Regarding claim 59, Shaatiel '487 teaches pharmaceutical composition (para [0063]) comprising the plant cell preparation of claim 43 and a pharmaceutically acceptable carrier (para [0063]).

SEE CONTINUATION SHEET.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V, Supplemental Page 1

Regarding claim 60, Shaatiel '487 teaches the use of the biologically active lysosomal enzyme of claim 37 for the manufacture of a medicament (para [0065], [0106]) for treating lysosomal storage disease (para [0020], [0064], Gaucher's disease).

Claims 16, 21, 24, 41, 45-47 and 49 lack an inventive step under PCT Article 33(3) as being obvious over Shaatiel '487.

Regarding claim 16, Shaatiel '487 teaches the cell of claim 13, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) is recombinantly produced (para [0030]) so as to have at least one core xylose (para [0024], [0310]) and at least one core alpha-(1,3) fucose (para [0308], [0310]). Although Shaatiel '487 does not specifically teach that the core xylose is linked in an alpha (1,2) glycosidic linkage, it would have been obvious to one of ordinary skill in the art that the xylose would be linked to the other sugars in an alpha (1,2) linkage because of the well known commonality of glycosylation enzymes systems in plant tissue (para [0139]) making alpha-(1,2) glycosidic linkages with xylose.

Regarding claim 21, Shaatiel '487 teaches the cell of claim 13, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) has at least one core xylose (para [0024], [0310]) and at least one core alpha-(1,3) fucose (para [0308], [0310]). As above, although Shaatiel '487 does not specifically teach that the core xylose is linked in an alpha (1,2) glycosidic linkage, it would have been obvious to one of ordinary skill in the art that the xylose would be linked to the other sugars in an alpha (1,2) linkage because of the well known commonality of glycosylation enzymes systems in plant tissue (para [0139]) making alpha-(1,2) glycosidic linkages with xylose.

Regarding claim 24, Shaatiel '487 teaches the human lysosomal protein of claim 23, wherein said xylose residue (para [0024]) is a core xylose residue (para [0024], [0310]). As above, although Shaatiel '487 does not specifically teach that the core xylose is linked in an alpha (1,2) glycosidic linkage, it would have been obvious to one of ordinary skill in the art that the xylose would be linked to the other sugars in an alpha (1,2) linkage because of the well known commonality of glycosylation enzymes systems in plant tissue (para [0139]) making alpha-(1,2) glycosidic linkages with xylose.

Regarding claim 41, Shaatiel '487 teaches the human lysosomal protein of claim 37, having an increased affinity (para [0049], increased affinity for target cells) for said macrophages (para [0234], [0235], promotes macrophage uptake), in comparison with the corresponding affinity of a naturally occurring lysosomal protein to other target cells (para [0049]). Although Shaatiel '487 does not specifically teach that the comparative affinity of the human lysosomal protein as taught by Shaatiel '487 is greater specifically for macrophages, it would have been obvious to one of ordinary skill in the art that enhanced macrophage uptake as taught by Shattiel '487 in macrophages (para [0234], [0235]) implies a greater binding affinity of the lysosomal protein for macrophages than native lysosomal protein would have been expected to exhibit in view of the increased capacity for of these proteins to their target cells in general (para [0049]).

Regarding claim 45, Shaatiel '487 teaches the plant cell preparation of claim 44, wherein said xylose residue (para [0024]) is a core xylose residue (para [0024], [0310]). As above, although Shaatiel '487 does not specifically teach that the core xylose is linked in an alpha (1,2) glycosidic linkage, it would have been obvious to one of ordinary skill in the art that the xylose would be linked to the other sugars in an alpha (1,2) linkage because of the well known commonality of glycosylation enzymes systems in plant tissue (para [0139]) making alpha-(1,2) glycosidic linkages with xylose.

Regarding claim 46, Shaatiel '487 teaches the plant cell preparation of claim 45, wherein said lysosomal protein (para [0067]) is a human glucocerebrosidase (para [0021], [0076]).

Regarding claim 47, Shaatiel '487 teaches the plant cell preparation of claim 46, wherein said human (para [0038], [0149]) lysosomal protein (para [0067]) comprises an amino acid sequence as set forth in SEQ ID NO: 8 (para [0077], claim 23, SEQ ID NO: 8).

Regarding claim 49, Shaatiel '487 teaches the plant cell preparation of claim 45, wherein said lysosomal protein (para [0067]) is a human (para [0038], [0149]) alpha-galactosidase (para [0038], [0046]).

Claims 3, 9, 35 and 50 lack an inventive step under PCT Article 33(3) as being obvious over Shaatiel '487 in view of US 2003/0077806 A1 to Selden et al. (hereinafter 'Selden '806').

Regarding claim 3, Shaatiel '487 teaches the isolated nucleic acid of claims 1 and 2, including a human (para [0038], [0149]) alpha-galactosidase (para [0038], [0046]) but does not teach that the alpha-galctosidase is the sequence in SEQ ID NO: 24. Selden '806 teaches an alpha-glactosidase comprising the sequence of SEQ ID NO: 24 (para [0014], Fig 6, SEQ ID NO: 4). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Selden '806 to utilize an isolated nucleic acid of a human alpha galactosidase of SEQ ID NO: 24, because the sequence of the nucleic acid of SEQ ID NO:4 as taught by Selden '806 would encode the exact alpha-galactosidase of Shaatiel '487 based on sequence identity.

Regarding claim 9, Shaatiel '487 teaches the isolated nucleic acid construct of claim 1, including a human (para [0038], [0149]) lysosomal protein (para [0067]) but does not teach that the human lysosomal protein is the sequence in SEQ ID NO: 24. Selden '806 teaches an alpha-glactosidase, a human lysosomal protein, comprising the sequence of SEQ ID NO: 24 (para[0014], [Fig 6, SEQ ID NO: 4). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Selden '806 to utilize an isolated nucleic acid of a human lysosomal protein of SEQ ID NO: 24, because the sequence of the nucleic acid of SEQ ID NO:4 as taught by Selden '806 would encode the exact human lysosomal protein of Shaatiel '487 based on sequence identity.

SEE CONTINUATION SHEET.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V, Supplemental Page 2

Regarding claim 35, Shaatiel '487 teaches the human lysosomal protein of claim 26, including a human glucocerebrosidase (para [0021], [0076]) but does not teach that the human cerbrosidase is the sequence in SEQ ID NO: 24. Selden '806 teaches a human cerbrosidase (para [0058]), comprising the sequence of SEQ ID NO: 24 (para [0014], [Fig 6, SEQ ID NO: 4). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Selden '806 to utilize an isolated nucleic acid of a human cerebrosidase of SEQ ID NO: 24, because the sequence of the nucleic acid of SEQ ID NO:4 as taught by Selden '806 would encode the exact human cerbrosidase of Shaatiel '487 based on sequence identity.

Regarding claim 50, Shaatiel '487 teaches the plant cell preparation of claim 49, including a human glucocerebrosidase (para [0021], [0076]) but does not teach that the human cerbrosidase is the sequence in SEQ ID NO: 24. Selden '806 as above teaches a human cerbrosidase (para [0058]), comprising the sequence of SEQ ID NO: 24 (para[0014], [Fig 6, SEQ ID NO: 4). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Selden '806 to utilize an isolated nucleic acid of a human cerebrosidase of SEQ ID NO: 24, because the sequence of the nucleic acid of SEQ ID NO:4 as taught by Selden '806 would encode the exact human cerbrosidase of Shaatiel '487 based on sequence identity.

Claims 4, 31, 32, 34, 48, 56 and 57 lack an inventive step under PCT Article 33(3) as being obvious over Shaatiel '487 in view of US 2005/0032211 A1 to Shaatiel (hereinafter 'Shaatiel '211').

Regarding claim 4, Shaatiel '487 teaches the isolated nucleic acid construct of claim 1, but does not specifically teach that said vacuolar targeting signal (para [0028], [0067]) is SEQ ID NO: 4. Shaatiel '211 teaches a vacuolar targeting signal (para [0221]) comprising the sequence of SEQ ID NO: 4 (SEQ ID NO: 4). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Shaatiel '211 to provide a vacuolar targeting signal comprising SEQ ID NO: 4 because the sequence taught by Shaatiel '211 (SEQ ID NO:4) is identical to the vacuolar targeting signal of SEQ ID NO: 4.

Regarding claim 31, Shaatiel '487 teaches the human lysosomal protein of claim 30, but does not specifically teach that said vacuolar targeting signal (para [0028], [0067]) is SEQ ID NO: 2. Shaatiel '211 teaches a vacuolar targeting signal (para [0221]) comprising the sequence of SEQ ID NO: 2 (para [0222], SEQ ID NO: 2). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Shaatiel '211 to provide a vacuolar targeting signal comprising SEQ ID NO: 2 because the sequence taught by Shaatiel '211 (para [0222], SEQ ID NO:2) is identical to the vacuolar targeting signal of SEQ ID NO: 2.

Regarding claim 32, Shaatiel '487 teaches the human lysosomal protein of claim 28, but does not specifically teach that said endoplasmic reticulum signal peptide (para [0023], [0028], [0128]) is SEQ ID NO: 1 or SEQ ID NO: 16. Shaatiel '211 teaches an endoplasmic reticulum signal peptide (para [0222]) comprising the sequence of SEQ ID NO: 1 (para[0222], SEQ ID NO: 1). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Shaatiel '211 to provide a vacuolar targeting signal comprising SEQ ID NO: 1 because the sequence taught by Shaatiel '211 (para [0222], SEQ ID NO:1) is identical to the endoplasmic reticulum signal peptide of SEQ ID NO: 1.

Regarding claim 34, Shaatiel '487 teaches the human lysosomal protein of claim 25, but does specifically teach that the human (para [0038], [0149]) lysosomal protein (para [0067]) comprises an amino acid sequence as set forth in SEQ ID NO: 15. Shaatiel '211 teaches a lysosomal protein (para[0028]) comprising the sequence of SEQ ID NO: 15 (SEQ ID NO: 14). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Shaatiel '211 to provide a humn lysosomal protein comprising SEQ ID NO: 15 because the sequence taught by Shaatiel '211 (SEQ ID NO:14) is identical to the human lysosomal protein of SEQ ID NO: 15.

Regarding claim 48, Shaatiel '487 teaches the plant cell preparation of claim 46, but does specifically teach that the human (para [0038], [0149]) lysosomal protein (para [0067]) comprises an amino acid sequence as set forth in SEQ ID NO: 15. Shaatiel '211 as above teaches a lysosomal protein (para[0028]) comprising the sequence of SEQ ID NO: 15 (SEQ ID NO: 14). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Shaatiel '211 to provide a humn lysosomal protein comprising SEQ ID NO: 15 because the sequence taught by Shaatiel '211 (SEQ ID NO:14) is identical to the human lysosomal protein of SEQ ID NO: 15.

Regarding claim 56, Shaatiel '487 teaches the plant cell preparation of claim 55, but does not specifically teach that said vacuolar targeting signal (para [0028], [0067]) is SEQ ID NO: 2. Shaatiel '211 as above teaches a vacuolar targeting signal (para [0221]) comprising the sequence of SEQ ID NO: 2 (para [0222], SEQ ID NO: 2). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Shaatiel '211 to provide a vacuolar targeting signal comprising SEQ ID NO: 2 because the sequence taught by Shaatiel '211 (para [0222], SEQ ID NO:2) is identical to the vacuolar targeting signal of SEQ ID NO: 2.

Regarding claim 57, Shaatiel '487 teaches the plant cell preparation of claim 55, but does not specifically teach that said endoplasmic reticulum signal peptide (para [0023], [0028], [0128]) is SEQ ID NO: 1 or SEQ ID NO: 16. Shaatiel '211 as above teaches an endoplasmic reticulum signal peptide (para [0222]) comprising the sequence of SEQ ID NO: 1 (para[0222], SEQ ID NO: 1). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Shaatiel '211 to provide a vacuolar targeting signal comprising SEQ ID NO: 1 because the sequence taught by Shaatiel '211 (para [0222], SEQ ID NO:1) is identical to the endoplasmic reticulum signal peptide of SEQ ID NO: 1.

SEE CONTINUATION SHEET.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of:
Box V, Supplemental Page 3

Claims 5 and 19 lack an inventive step under PCT Article 33(3) as being obvious over Shaatiel '487 in view of WO 2007/005882 A2 to Weisssinger et al. (hereinafter "Weissinger").

Regarding claim 5, Shaatiel '487 teaches the isolated nucleic acid construct of claim 2 but does not specifically teach that the endoplasmic reticulum retention signal (para [0023], [0028], [0128]) is SEQ ID NO:23 (KDEL). Weissinger teaches SEQ ID NO:23 (KDEL) for endoplasmic reticulum targeting (pg 5, ln 21-23, Fig 5, SEQ ID NO:4) for expressing foreign genes in plants (pg 1, ln 12-14). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Weissinger to use a KDEL peptide (SEQ ID NO:23) as an endoplasmic reticulum retention signal, because the peptide KDEL functions in the same capacity for endoplasmic reticulum retention as taught by Weissinger.

Regarding claim 19, Shaatiel '487 teaches the cell of claim 17 but does not specifically teach that the plant cell (para [0139]) is a tobacco cell. Weissinger teaches expression of foreign genes in plants (pg 1, ln 12-14) wherein the plants comprise tobacco cells (pg 4, ln 10-15). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and Weissinger to utilize tobacco cells for the expression of polynucleotides encoding lysosomal proteins, because the use of tobacco cells for the expression of similar heterologous genes as taught by Weissinger makes them an exemplary candidate for lysosomal protein production.

Claim 39 lacks an inventive step under PCT Article 33(3) as being obvious over Shaatiel '487 in view of US 2005/0281805 A1 to LeBowitz et al. (hereinafter "LeBowitz").

Regarding claim 39, Shaatiel '487 teaches the human lysosomal protein of claim 37 but does not specifically teach that the said biological activity (of the lysosomal protein) (para [0067]) is uptake into fibroblasts. LeBowitz teaches teaches uptake of modified alpha galactosidase for treatment of Fabry's disease (para [0160], [0199]) in fibroblasts (para [0049], [0166], [0167], [0175]). It would have been obvious to one of ordinary skill in the art to combine the teachings of Shaatiel '487 and LeBowitz to test for fibroblast uptake enhancement of lysosomal proteins such as alpha-galactosidase based on the teaching LeBowoitz which relates fibroblast uptake with treatment of Fabry's disease, a genetic disease resulting from alpha galctosidase deficiency.

Claims 6, 7, 36 and 51 meet the criteria set out in PCT Article 33(2)-33(3) because the prior art does not teach or clearly suggest the claimed subject matter.

Regarding claim 6, Shaatiel '487 teaches the isolated nucleic acid construct of claim 2, but does not specifically teach that the construct comprises SEQ ID NO:19. WO 2008/132743 A2 to Shaatiel et al. (hereinafter "Shaatiel '743") teaches SEQ ID NO:19 (SEQ ID NO: 19), but since Shaatiel '487 was published after the priority date of the present claim is not prior art; the prior art neither teaches nor suggests the nucleic acid construct comprising SEQ ID NO: 19.

Regaridng claim 7, Shaatiel '487 teaches the isolated nucleic acid construct of claim 2, but does not specifically teach that the construct comprises SEQ ID NO:17. Shaatiel '743 teaches SEQ ID NO:17 (SEQ ID NO: 17), but since Shaatiel '487 was published after the priority date of the present claim is not prior art; the prior art neither teaches nor suggests the nucleic acid construct comprising SEQ ID NO: 17.

Regarding claim 36, Shaatiel '487 teaches the human lysosomal protein of claim 26 but does not specifically teach that the human (para [0038], [0149]) lysosomal protein (para [0067]) comprises an amino acid sequence as set forth in SEQ ID NOs: 18 or 20. US 6,083,725 A to Selden et al. (hereinafter "Selden '725") teaches a sequence having 93% homology to SEQ ID NO: 18. US 2002/0088024 A1 to Garger et al. (hereinafter "Garger") teaches a sequence having 92% homology to SEQ ID NO: 20. However, since neither Selden '725 nor Garger teaches the specific sequence of SEQ ID NO: 20 or SEQ ID NO: 18, the prior art neither teaches nor suggests the lysosomal protein comprising SEQ ID NOS: 18 or 20.

Regarding claim 51, Shaatiel '487 teaches the plant cell preparation of claim 49, but as above does not specifically teach that the human (para [0038], [0149]) lysosomal protein (para [0067]) comprises an amino acid sequence as set forth in SEQ ID NOs: 18 or 20. Selden 725 as above teaches a sequence having 93% homology to SEQ ID NO: 18. Gargeras above teaches a sequence having 92% homology to SEQ ID NO: 20. However, since neither Selden '725 nor Garger teaches the specific sequence of SEQ ID NO: 20 or SEQ ID NO: 18, the prior art neither teaches nor suggests the lysosomal protein comprising SEQ ID NOS: 18 or 20.

Claims 1-60 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.